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Antioxidant Potential of Black, Greenand Oolong Tea Methanol Extracts

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Abstract

Degenerative diseases and chronic diseases are often caused by oxidative stress. Oxidative stress caused by free radicals. Antixodant as inhibitor are needed to prevent it which is one of antioxidant sources is tea. Tea processing generally produce various kinds of teas such as black, green and oolong tea. Tea processing affect the content of phenolic compounds. The aim of the research is to evaluate phytochemical content, total phenolic content of black tea, green tea and oolong tea extracts using catechin, quercetin, kaempferol, myricetin as standard, and to evaluate the antioxidative potency of black tea, green tea and oolong tea extracts compared to catechin, quercetin, kaempferol, myricetin. Phytochemical assay using modified Farnsworth method, the antioxidant activity were measured by its 1,1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging activity. Green tea extract contained highest phenolic and flavonoid. The highest antioxidant activity was green tea extract with IC_{50}=0,487 µg/mL. Green tea extract content phenol and flavonoid are higher compared to the other extracts, green tea extract has the highest antioxidant activity.

Keywords: antioxidant, black tea extract, green tea extract, oolong tea extract, total phenolic content

Introduction

Various degenerative and chronic diseases are often caused by oxidative stress. Oxidative stress is caused by free radicals. It highly reactive and unstable because of its unpaired electron in the outer atomic orbital. Free radical can react with the cells molecules by binding to it. It can oxidize the nucleic acid, proteins, fats and even the cells DNA and initiate the degenerative disease (Halliwell & Gutteridge, 2007). Free radicals are derived either from normal essential metabolic process in the human body or from external exposure (Bagchi & Puri, 1998). The antioxidants as oxidation inhibitor are needed to overcome the negative effect of the free radicals. Antioxidant inhibits the oxidation by reacting to reactive free radicals to form reactive substances that relatively stable. Endogenous antioxidant already present in the human body. However the exogenous antioxidant still needed if the free radicals present in copious amounts (Johnson, 2002). A balance between free radicals and antioxidant is necessary for proper physiological function. Antioxidant may exert their effect on biological systems by different mechanism, including electron donation, metal ion chelation, co-antioxidants, or by gene expression regulation (Krinsky, 2000; Lobo et al., 2010).

There are two kinds of antioxidant based on its source, natural antioxidant and synhetic antioxidant. Synthetic antioxidants are carcinogenic when it consumed for long term. The needs of natural antioxidant that have fewer side effect and less toxic continues to rise.Natural antioxidants can protect the body against the damage caused by reactive oxygene species (ROS), inhibit the degenerative disease and inhibit the lipid peroxidase activity (Wiseman et al., 1997). Tea-one of the most popular beverage- components possess antioxidant activity.Most commercially prepared tea is obtained form the leaf of the plant *Camelia sinensis* (Satoh et al., 2005). Among tea, 69% of consumption is black tea, 28% of consumption is green tea and 3 % others is oolong tea (Cabrera et al., 2008). Teas of *C.sinensis* undergo different manufacturing processes. Green tea is produced by steaming (Japan) or panning (China) to prevent cathechin oxidation by polyhenol oxidase. Oolong tea is semi-fermented while black tea is fully fermented (Eric et al., 2011). Many studies have shown that green, black and oolong tea has antioxidant properties (Xie et al., 1993; Wiseman, 1997; McKay & Blumberg, 2002; Zhu et al., 2002; Higdon & Frei, 2003; Satoh et al., 2005). Tea processing will affect the phenol content and ultimately will affect the antioxidant activity of it (Higdon & Frei, 2003). Therefore, it become important to investigate the antioxidant activity of the extract of black tea, green tea, and oolong tea and test the phenol content based on the cathechin, quercetin, kaempferol and myricetin standard.

Methodology

Plant material preparation, extraction and phyrochemical content assay

Dried leaves green tea and black tea were obtained from Cisaruni Plantation, PTPN VIII, West Java, Indonesia

and dried oolong tea was obtained from Tea Plantation, East Java. Extraction was performed based on maceration method using methanol 96% as the solvent (Widowati et al., 2011a; Widowati et al., 2013a; Widowati et al., 2014a; Widowati et al., 2014b). The extraction yielded 20.139 % of black tea, 22.11 % of green tea, and 17.39 % of oolong tea. The green tea, black tea, and oolong tea extract were tested by phytochemical assay using modified Fransworth method including terpenoid, phenol, steroid, triterpenoid, flavonoid, tannin, alkaloid, and saponin assay (Fransworth *et al*., 1966; Widowati et al., 2010; Bera et al., 2014).

Total Phenol Assay

Briefly 25 µL standard solution in 10 concentration level (500; 250; 125; 62,5; 31,25; 15,625; 7,81; 3,95; 1,98; and 0,98 µg/mL) of catechin, quercetin, kaempferol, and myricetin and sample (extract from green tea, black tea, and oolong tea) in concentration of 500 µg/mL were prepared for total phenol assay. Each standard and sampled was mixed with 125 µL follin 10% and 100 µL of Na_2CO_3 7,5 % in microplate. The reaction then incubated at 45°-50°C for 10 minutes. The absorbance was measured in 760nm of wavelength using microplate reader. The linear regression equation ($y= a + b$) was created based on the standard (cathechin, quercetin, kaempferol, myricetin) absorbance value. The analysis of phenol content of sample was performed based on the each of standard linear regression equation (Ivanova et al., 2005; Widowati et al., 2011a; Widowati et al., 2015).

DPPH scavenging activity assay

DPPH (2,2-diphenyl-1-picrylhydrayl) free radical scavenging activity were analyzed based on the linear regression equation continued by median Inhibitory Concentration (IC_{50})value determination. Ten concentration levels (500; 250; 125; 62,5; 31,25; 15,625; 7,8; 3,9; 1,9; and 0,9 µg/mL) of black tea, green tea,and oolong tea extract as working solution was prepared for this assays. Briefly, 50µL samples (working solution) were added to a microplate followed by 200µL DPPH (Sigma-Aldrich) solution (0,077 mmol/L in methanol). The reaction was shaken vigorously and kept in the dark for 30 minutes at room temperatures. DPPH scavenging activity was determined by microplate reader at 517 nm. Metanhol absolute was used as blanko. The IC_{50} value then determined. The radical scavenging activity of each sample was measured according to equation 1 (Han et al., 2004; Widowati et al., 2013b; Widowati et al., 2013c; Widowati et al., 2015).

$$S \qquad \% = (A_c - A_s)/(A_c \times 100) \qquad (1)$$

Description
A_s = sample absorbance
A_c = negative control absorbance (without sample)

Results

Phytochemical content of tea extracts

Table 1 shows the different phytochemical content, including terpenoid, phenol, steroid, triterpenoid, flavonoid, tannin, alkaloid, and saponin of green tea, black tea, and oolong tea methanol extract. All of phytochemical tested was found in the green tea extract. High level of phenol also found in green tea and oolong tea extract. Green tea also containts the highest level of alkaloid compared to the other tea extract. Otherwise, steroid and alkaloid did not observed in the black tea extract. Triterpenoid and tannin also not found in the oolong tea extract. Less content of saponin also found in all of the extract.

Table 1. Phytochemical content assay of black tea, green tea, and oolong tea methanol extract.

Sample	Phytochemical assay							
	Terpenoid	**Phenol**	**Steroid**	**Triterpenoid**	**Flavonoid**	**Tannin**	**Alkaloid**	**Saponin**
Black tea	++	++	-	+	++	+	-	+
Green tea	+	++++	+	+	+++	+	+++	+
Oolong tea	+	++++	++	-	+++	-	+	+

Description:
++++ : very high content, +++: high content, ++: moderate content, +: low content, - : undetected

Phenolic compound of tea extracts

Statistical assay based on linear regression equation was performed to analyze the total phenol of black tea, green tea, and oolong tea based on the standard solution (cathechin, quercetin, kaempferol and myricetin). The highest level of total phenol was showed by green tea extract (Table 2). The degree of oxidation affected by the polyphenols profile of the tea. (Balentine et al., 1997).

Table 2. The average of total phenol level concentration of black tea, green tea, and oolong tea (µg/mg).

Sample	Catechin Equivalent (CE)	Quercetin Equivalent (QE)	Kaempferol Equivalent (KE)	Myricetin Equivalent (ME)
Black tea extract	14,33	4,50	4,33	4,17
Green tea extract	23,33	9,71	4,33	6,17
Oolong tea extract	16,08	5,09	3,33	4,67

DPPH Scavenging Activity

The most active extract in DPPH scavenging activity showed by green tea extract indicated by the lowest IC_{50} value compared to the other extract and standard (Table 3). DPPH is a stable free radical because of the unpaired electron. The unpaired electron of the radical becomes paired in the presence of hydrogen donor, decreasing the absorption in 517 nm of wavelength. The DPPH scavenging activity has been widely used to test the compound ability as a free radical scavenger and antioxidant activity in food or plat extract (Satoh et al., 2005). Previous study already obtained that the black tea has the antioxidant activity through DPPH free radical scavenging activity with IC_{50}= 5,405 µg/mL (Widowati et al., 2011a), catechin (C) diluted in methanol with IC_{50} =8.11 µM (Evacuasiany et al., 2014) and catechin diluted in DMSO with IC_{50} =7.02 µg/ml (Budiman et al., 2014).

Table 3. *Inhibitory Concentration* (IC_{50}) Value of Antioxidant DPPH Scavenging Activities of Tea extract and Standard.

Samples	Replication	Linear equation	R^2	IC_{50} (µg/ml)	Average IC_{50}
Green tea extract	1	Y= 4,299x + 48,70	0,767	0,30	0,487 ± 0,258
	2	Y= 4,539x + 46,45	0,735	0,78	
	3	Y= 4,342x + 48,37	0,751	0,38	
Oolong te extract	1	Y= 7,363x + 12,93	0,911	5,03	5,005 ± 0,060
	2	Y= 7,225x + 13,55	0,918	5,04	
	3	Y= 7,286x + 14,03	0,911	4,94	
Quercetin	2	Y= 6,592x + 3,575	0,989	7,04	4,279 ± 0,065
	3	Y= 6,666x + 3,671	0,987	6,95	
	1	Y= 6,153x + 23,65	0,901	4,28	
	2	Y= 6,095x + 24,32	0,893	4,21	
	3	Y= 6,128x + 23,39	0,878	4,34	
Kaempferol	1	Y = 8,731x - 11,662	0,823	7.06	7.154±0.134
	2	Y = 8,063x - 8,924	0,887	7.31	
	3	Y = 9,039x - 14,107	0,827	7.09	
Myricetin	1	Y = 12,803x - 6,749	0,829	4,43	4,496±0,058
	2	Y = 11,588x - 4,270	0,809	4,69	
	3	Y = 10,89x + 2,464	0,719	4,37	

Discussion

Tea is one of the most widely consumed beverages in the world which is grouped into three main groups, including green, black, and oolong tea, according to the fermentation process, Fermentation of tea can affect the phytochemical content of different tea extract confirmed by the result that highest phenol and flavonoid content was observed in green tea (non fermented tea) and oolong tea (semi fermented tea) compared to black tea (fully fermented tea). In the fermentation process, the catechin is oxidized resulting thearubigin, theaflavin including theaflavin (TF1), theaflavin-3-gallate (TF2A), theaflavin-3'-gallate(TF2B), and theaflavin-3,3'-digallate (TF3B) which is the key component of the black tea flavour (Leung et al., 2001;. Yang & Landau, 2002; USDA, 2003). Phytochemical assay of green tea extract Catechins are a group of natural polyphenols found in green tea. Previous study of green tea exract using ehanol 70% resulted phenols (+++), triterpenoids (++), steroid (-), terpenoids (+++), saponins (+), alkaloids (++), flavonoids (++), tannins (+++) (Fanny et al., 2015). Different solvent concentration resulted different compounds, yield and bioacivities (Tiwari et al., 2011) The different solvent would result the different compound and bioactivity (Pujimulyani et al., 2004; Widowati et al., 2011b)., validated by previous study that water extract of Forsythia koreana flowers exhibited a higher phenolic content than ethanol extract (Yang and Kang, 2011). Otherwise, theaflavins are another group of polyphenol

pigment found in both black and oolong teas.Oolong tea that partially oxidized and retains a considerable amount of the original catechin (Nor & Mohd, 2003). Many studies have demonstrated that both catechin and theaflavin have strong free-radical-scavenging activity both *in vivo* and *in vitro* (Lai et al., 2001).

The chemical composition of tea includes proteins, polysaccharides, polyphenols (cathecins or flavan-3-ols, theaflavins, thearubigins, and proanthocyanidins), chlorophyll, minerals, and trace elements volatile compounds, amino and organic acids, lignins and alkaloid (Chen et al., 2009). Tea is wellknown for it health benefits because of its poliphenol bioactive content, especially chatechin which have antioxidants activity that play a role in reducing the free radical effect (Vecchia et al., 1992; Bravo et al., 1998; Nagao et al., 2005). Catechin belong to flavonoid that posses the high antioxidant activity in biological system. (Gramza et al., 2005; Evacuasiany et al., 2014)

According to the Table 2. the most active extract in DPPH scavenging activity was the green tea extract showed by the lower of IC_{50} values compared to the catechin, quercetin, kaempferol, and myricetin. The green tea extracts have the higher antioxidant activity through DPPH scavenging activity and IC_{50} value compared to the black tea extracts (Widowati et al., 2011a). That result is in accordance to the phytochemical assay (Table 1) and total phenol assay (Table 2) showed that the green tea extract containts the higher total phenol content compared to black tea and oolong tea extracts and the higher flavonoid compared to the black tea extracts. High correlations were observed between antioxidants activities and polyphenol phytochemicals content. The antioxidants activities most probably might be contributed by polyphenols contents in the plant extracts (Bakar et al., 2009; Ling et al., 2010; Nor & Mohd, 2013).

The result of this study also confirmed that the green tea extract posses the higher antioxidant activity compared to the catechin, quercetin, kaempferol and myrietin. Table 2 also confirmed that teas extract contain catechin and various compound including quercetin, kaempferol and myricetin (Cabrera et al., 2006). Some research also investigated that black tea, oolong tea and green tea posses the antioxidant activity (Xie et al., 1993; Zhu et al., 2002). This study also in line with the research that polyphenol compound have antioxidant activity (Higdon & Frei, 2003; Widowati et al., 2011a). Green tea is the good source of polyphenol especially for flavanol and flavonol compounds which is found in 30% of tea leaf dry mass (Wolfram et al., 2008). The antioxidant activity of green tea extracts was higher than oolong tea and black tea extracts (Gramza et al., 2005). Satoh *et al* (2005) also found that the green tea has the highest percent DPPH scavenging activity followed by roasted tea, oolong tea, and black tea respectivelly. That antioxidant activities have a strong correlation with the total phenolic content. The oxidation of flavanol in green tea may greatly contribute to the high antioxidant activities compared to the other kinds of tea (Von et al., 2000; Satoh et al., 2005).

Conclusion

The green tea metanhol extract have the higher total phenol content compared to the other extracts (black tea and oolong tea). Green tea extract aslo show the highest antioxidant DPPH scavenging activity. Hence, the need to exploit the potential of green tea in pharmaceutical industries arises.

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